EXPERIMENT 7B

Spectrophotometric Determination of the Copper (II) Sulfate Pentahydrate Content in a Mixture

QUANTITATIVE ANALYSIS

Quantitative analysis is a branch of analytical chemistry concerned with the amounts of the various components within a sample. Although the division is somewhat inadequate, the methods of quantitative analysis are often separated into three categories: gravimetric, volumetric, and instrumental. The basic distinction between the first two types and the third is the nature of the equipment used in the laboratory: noninstrumental work relies on traditional items such as crucibles, burets, volumetric flasks, and pipets while an instrumental approach requires sophisticated devices such as spectrometers.

SPECTROPHOTOMETRIC ANALYSIS PRINCIPLES

Spectrophotometric analysis is a type of instrumental analysis that involves the interaction of electromagnetic radiation with matter. The most common regions of the electromagnetic spectrum used for analysis are the ultraviolet, visible, and infrared, although other regions are also used.

Every chemical substance possesses its own unique set of electronic, vibrational, and rotational energy levels, in much the same way that each person has a distinct set of fingerprints. When electromagnetic radiation falls incident upon an atom, ion, or molecule, the radiation absorbed is of an energy equal to the difference between two energy levels. This places the atom, ion, or molecule in an excited state. The remainder of the electromagnetic radiation passes through the sample (the transmitted light) and an electromagnetic radiation detector detects it. The absorbed energy, $E$, is related to the wavelength, $\lambda$, of the electromagnetic radiation:

$$E = \frac{hc}{\lambda}$$

Where $h$ is Planck’s constant and $c$ is the speed of light. When a substance absorbs light in the visible region, it is usually an electron that is raised from a lower to a higher energy state. When white light (electromagnetic radiation containing all wavelengths of visible light) passes through a sample, the wavelengths of light absorbed by the sample excite electrons in the sample. Wavelengths of light not absorbed pass through the sample, reach our eyes, and our eyes detect the wavelengths of visible light not absorbed. Therefore, the color we see is complimentary to the one absorbed. A color wheel, which shows the colors of the rainbow, is shown to the right. On a color wheel, the complementary color is the color found directly opposite on the wheel.

If the sample absorbs energy from the violet region, the transmitted light appears yellow, and the higher the concentration of violet absorbing material, the more intense is the yellow. Thus the eye, one kind of electromagnetic radiation detector, only detects the transmitted light. The table on the next page lists the colors corresponding to wavelength regions of light (and their compliments) in the visible region of the electromagnetic spectrum.
Color and Wavelength in the Visible Region of the Electromagnetic Spectrum

<table>
<thead>
<tr>
<th>Color Absorbed</th>
<th>Wavelength Absorbed (nm)</th>
<th>Color Transmitted (Observed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red</td>
<td>630-750</td>
<td>Green</td>
</tr>
<tr>
<td>Orange</td>
<td>595-630</td>
<td>Blue</td>
</tr>
<tr>
<td>Yellow</td>
<td>580-595</td>
<td>Violet</td>
</tr>
<tr>
<td>Green</td>
<td>500-580</td>
<td>Red</td>
</tr>
<tr>
<td>Blue</td>
<td>435-500</td>
<td>Orange</td>
</tr>
<tr>
<td>Violet</td>
<td>380-435</td>
<td>Yellow</td>
</tr>
</tbody>
</table>

The one specific wavelength at which a sample has its maximum absorption is symbolized by $\lambda_{\text{max}}$. For a yellow substance, $\lambda_{\text{max}}$ will be in the violet region, somewhere between 380 nm and 435 nm.

Measurements of the amount of light a colored substance absorbs are made using an instrument called a spectrometer. The spectrometer measures light intensities with a photosensitive cell at specific (but variable) wavelengths. The spectrometer shines an incident light of known intensity, $I_o$, on the aqueous sample, whose container width is $\ell$. The intensity of the transmitted light that passes through the sample, $I_t$, is measured with the photosensitive cell.

The **transmittance**, $T$, of the solution is defined as the fraction of the incident light that passes through the sample, which mathematically is the ratio of the transmitted light to the incident light:

$$ T = \frac{I_t}{I_o} $$

For example, if the intensity of the incident light on a sample is 25 watts, and the intensity of the transmitted light is 15 watts, then the transmittance is

$$ T = \frac{15 \text{ watts}}{25 \text{ watts}} = 0.60 $$

The **absorbance**, $A$, of the solution is the negative logarithm of the transmittance:

$$ A = -\log T $$

Therefore the absorbance for this sample would be

$$ A = -\log (0.60) = 0.22 $$

The absorbance of a sample is usually measured at the wavelength where the detection of the colored substance in the solution is the most sensitive, which is the wavelength of maximum absorbance, $\lambda_{\text{max}}$. 

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At a specific wavelength, such as $\lambda_{\text{max}}$, three factors control the amount of light that a sample absorbs:

1. **Concentration**, $c$, of the absorbing species. The higher the concentration of the absorbing material, the greater the absorbance.

2. **Path length**, $\ell$, or distance the incident light must pass through the sample. This is determined by the width of the spectrophotometric cell, and the further light must travel through a solution, the greater the absorbance.

3. **Extinction coefficient**, $\varepsilon$, or the probability of the absorbing substance to absorb photons of a specific wavelength. This quantity is different for each absorbing substance, and it is different for each wavelength absorbed.

The measured absorbance, $A$, of a sample is directly proportional to these three quantities through the following relationship:

$$A = \varepsilon \ell c$$

The equation is commonly referred to as the **Beer-Lambert Law**. Because this relationship matches the general equation for a line

$$y = mx + b$$

if a graph is made in which the absorbances of different samples ($A$) are plotted against the concentrations of the absorbing material in each sample ($c$), the graph will be a line, the slope will be equal to $\varepsilon \ell$, and the y-intercept will be equal to 0.

**INTRODUCTION**

In this experiment, the amount of copper (II) sulfate pentahydrate impurity found in the potassium nitrate crystals from Experiment 7A will be determined spectrophotometrically. The copper (II) ions in the copper (II) sulfate pentahydrate impurity, when dissolved in an ammonia-water solution, produce the complex ion $\text{Cu(NH}_3\text{)}_4^{2+}$, which has a deep blue color. The color is due to the absorption by the complex ions of part of the visible spectrum, with the strongest absorption at wavelengths between 595 nm and 630 nm.

Ammonia-water solutions of known concentrations of copper (II) sulfate pentahydrate have been prepared. These solutions will each be placed in the same spectrophotometric cell, and their absorbances measured at the same wavelength, $\lambda_{\text{max}}$, the one in which the maximum absorption occurs. Because $A$ and $c$ are directly proportional, graphing absorbance versus concentration for these solutions will produce a straight line (with the slope of the line equal to $\varepsilon \ell$). The line that is produced is called a **Calibration Line**. This calibration line will allow us to determine the concentration of copper (II) sulfate pentahydrate in the solution from the Experiment 7A crystals by correlating the absorbance of that solution to its corresponding concentration.

**PROCEDURE**

1. Students will work individually for this experiment. Except for the laboratory handout, remove all books, purses, and such items from the laboratory bench top, and placed them in the storage area by the front door. For laboratory experiments you should be wearing closed-toe shoes. Tie back long hair, and do not wear long, dangling jewelry or clothes with loose and baggy sleeves. Open you lab locker. Put on your safety goggles, your lab coat, and gloves.
CONTINUED FROM EXPERIMENT 7A

2. If your black silicon carbide was drying in your lab drawer, remove the beaker with the black silicon carbide crystals from your laboratory locker, and using your analytical balance, determine the mass of the beaker, filter paper and silicon carbide crystals. Complete your calculations for Experiment 7A, and resubmit your Experiment 7A lab report.

3. Place your filter paper and silicon carbide crystals in the wide-mouth waste bottle in Fume Hood A.

SPECTROPHOTOMETRIC ANALYSIS

4. Remove the beaker with the white potassium nitrate crystals from your laboratory locker. Before weighing anything on the balance, press one of the tare keys with the symbol "$\rightarrow T/0 \leftarrow$" and make sure the display reads "0.000 g. Carefully open the side sliding door of the balance. Watch for debris in the track of the sliding door and clean it out if it is present because the sliding door is one of the most fragile parts of the balance. Place the 50-mL beaker on the balance pan and close the door. When the display shows a mass with three places past the decimal point followed by a "g" for grams, press a tare key again to zero-out the mass of the beaker.

5. Remove the beaker from the balance and then add as close to 1.000 grams of your potassium nitrate crystals to the beaker as possible.

NOTE: If any crystals are spilled on the balance or on the lab bench, clean them up immediately, and dispose of them in the solid waste container in the fume hood. If there are any crystals left on the balance or the lab bench at the end of the lab period, the instructor will deduct one point from everyone's lab score as a charge for cleaning up after you.

6. Dissolve the crystals in 6.0 mL of deionized water, and then add 6.0 mL of 6 M ammonia.

CAUTION: Ammonia (also called ammonium hydroxide) is corrosive and an irritant.

This should give you slightly more than 12 mL of solution. Because of the presence of the ammonia, if there are any copper (II) ions in the solution, they will form a blue complex ion.

7. If the laptop computer is turned on, proceed to step 8. If the computer is off, turn it on, and when the Log On dialogue box appears, for User Name: type in your Saddleback (or IVC) email address, and for Password: type in your Saddleback (or IVC) password. If any other dialogue boxes appears, select No or Cancel or Close. Double click on Logger Pro. If a Setup Interface dialogue boxes appears, under Port: select LabPro-USB, then select OK. The screen should now show the visible spectrum.

8. Obtain a cuvet from your lab locker. Handle the cuvet by the two ribbed sides. Any fingerprints or foreign material on the two clear slides of the cuvet affects the intensity of the transmitted light. Fill the clean cuvet three-quarters full with deionized water. This is the blank, a solution that is 0% copper (II) sulfate, and should not absorb any of the incident light from the spectrometer.

9. Use a grease pencil (found in a drawer at the back of the lab room) to make a small mark on the top of the cuvet so that you can place it into the spectrometer in the same orientation throughout the experiment. From the Experiment menu, Select Calibrate ► Spectrometer. The calibration dialog box will display the message: “Waiting . . . seconds for lamp to warm up.” The minimum warmup time is one minute. When the dialogue box appears that says “Place a blank cuvet in the device”, wipe the cuvet with a KimWipe and place it in the spectrometer so that the beam of light passes through to two clear sides. Click Finish Calibration, and when the warm-up is complete click OK.
10. Remove the cuvet from the spectrometer and empty the deionized water into the sink.

11. Obtain the four standard copper (II) sulfate pentahydrate solutions. Select the most concentrated standard solution, Solution D. Condition the cuvet by rinsing it three times with 5-drop portions of Solution D, holding the cuvet in a horizontal position and rolling it and/or tilting it back and forth to make sure that the solution wets the entire inside surface. Empty the solution into a waste beaker on your lab bench. This is done to remove any contaminants or drops of water from the cuvet that would dilute your standard solution. Fill the cuvet three-quarters full with Solution D. Wipe the cuvet with a Kimwipe, removing water and fingerprints.

12. Place the cuvet into the spectrometer. Click [Collect]. A full spectrum graph of the solution will be displayed. Click [Stop] to complete the analysis.

13. Click the **Configure Spectrometer Data Collection** button (the one with the colored graph on it), from the toolbar. A dialog box will appear. Under **Collection Mode**, select **Abs vs. Concentration**. Under **Column Name**: type “Percent Copper (II) Sulfate Pentahydrate”, under **Short Name**: type “% CuSO4”, and under **Units**: type “%”. Note that one area of the graph contains the wavelengths where the blue solution absorbs light the strongest. The one wavelength that is absorbed strongest is called the wavelength of peak absorbance, \( \lambda_{\text{max}} \). The wavelength of peak absorbance should be automatically selected with a check in the box next to it. If it is not, choose the \( \lambda_{\text{max}} \) by clicking on the graph or by checking the box next to the desired wavelength. Record this value in your Data Table. Click [OK], then click **Yes** to store the latest run.

14. Leave the cuvet in the spectrometer. Record the mass percent of the copper (II) sulfate pentahydrate in Solution D (which is found on its bottle or on the front board) in your Data Table. Click [Collect]. When the absorbance reading stabilizes (after about 5 seconds), click [Keep]. **DO NOT CLICK STOP!** A dialogue box in LoggerPro will now appear. Enter the mass percent of the copper (II) sulfate pentahydrate for Solution D into the dialogue box and click [OK]. Record the spectrometer’s absorbance value for Solution D in your Data Table. **DO NOT CLICK STOP!** Remove the cuvet from the spectrometer and discard the cuvet contents into the waste beaker on your lab bench.

15. Obtain the second most concentrated standard copper (II) sulfate pentahydrate solution, Solution C. Record the mass percent of the copper (II) sulfate pentahydrate in Solution C in your Data Table. Condition the cuvet three times with 5-drop portions of Solution C, each time emptying the cuvet’s contents into the waste beaker, and fill the cuvet three-quarters full with Solution C. Wipe the cuvet with a Kimwipe, and place it in the spectrometer. When the absorbance reading stabilizes (after about 5 seconds), click [Keep]. **DO NOT CLICK STOP!** Enter the mass percent of the copper (II) sulfate pentahydrate for Solution C into LoggerPro and click [OK]. Record the spectrometer’s absorbance value for Solution C in your Data Table. **DO NOT CLICK STOP!** Remove the cuvet from the spectrometer and discard the cuvet contents in a waste beaker on your lab bench.

16. Repeat step 15 for the third copper (II) sulfate standard solution, Solution B, the fourth copper (II) sulfate standard solution, Solution A, and finally a blank (deionized water), which is 0% copper (II) sulfate. After the blank you have now finished testing all of the standard solutions. Now, click [Stop].

17. Make sure that the graph with your five data points is selected. To determine the best-fit line equation for the calibration line of the standard solutions, click the **Linear Fit** button (the one with the \( \mathbf{R} \) on it) on the toolbar. A dialogue box will appear that contains the numerical value for the slope, \( m \), which equals \( \varepsilon \) from the Beer-Lambert Law, as well as the numerical value for the y-intercept, \( b \), which should be theoretically 0 from the Beer-Lambert Law. Write down the slope and the y-intercept for the calibration line in your Data Table, indicating the proper number of significant figures based upon the concentration values you graphed.
18. The dialogue box will also display a *correlation coefficient* (labeled as correlation) which indicates the strength of the linear relationship between the two plotted variables. A correlation coefficient of 1.000 means the data points lie on a perfectly straight line. The closer your correlation coefficient is to 1.000, the stronger the linear relationship between absorbance and concentration, and the better your calibration line. Write down the correlation coefficient for the calibration line in your Data Table.

19. Before testing the crystal solution from Experiment 7A, the spectrometer can be recalibrated to insure that the zero readings has not drifted over time. Condition the cuvet three times with 5-drop portions of deionized water, each time emptying the cuvet’s contents into the sink. Then, fill the clean cuvet three-quarters full with deionized water. From the *Experiment* menu, Select *Calibrate ► Spectrometer*. When the dialogue box appears that says “Place a blank cuvet in the device”, click *Skip Warmup*, then wipe the cuvet with a KimWipe and place it in the spectrometer so that the beam of light passes through to two clear sides. Click *Finish Calibration*, and when the calibration is complete click **OK**. Remove the cuvet from the spectrometer and empty the deionized water into the sink.

20. Condition the cuvet three times with 5-drop portions of your crystal solution from Experiment 7A, each time emptying the cuvet’s contents into the waste beaker on your lab bench. Fill the cuvet three-quarters full with the crystal solution. Wipe the cuvet with a Kimwipe, and place it in the spectrometer. When the absorbance reading stabilizes (after about 5 seconds), record the absorbance value for the crystal solution in your Data Table.

21. Calculate the mass percent of copper (II) sulfate pentahydrate in the crystal solution from the Beer-Lambert Law, using the measured absorbance of the crystal solution and then the slope and y-intercept from your calibration line.

22. Click on the *Autoscale Graph* button [A] (the one with the A on it) from the toolbar to autoscale the graph. Arrange any floating boxes so they are not covering each other, or covering up any part of your data. To save your data, from the menu bar select *File*, then *Save*, and from the left column select *Desktop*. Save your data as *Exp 7B, Your Name*.

23. In Logger Pro, from the *File* menu, select *Print*, click *Print Footer*, type your name, and click **OK**. The selected printer should be *ISC1323000A*. Click **OK** to print, and your graph will be sent to the printer in the lab room and printed there. Retrieve your graph and attach it to your lab report.

24. From the menu bar on the laptop, select *Data*, then *Clear All Data*. Close each of the floating dialogue boxes remaining on the graph by clicking the X in the upper left hand corner of each of the floating dialogue boxes.

25. Rinse the cuvet with deionized water and shake dry, but do not dry with either paper towels which can leave lint. Return the cuvet to your lab locker. *Any remaining crystals should be disposed of in the solid waste container, located in Fume Hood A. Any remaining solutions should be disposed of in the liquid waste container, also located in Fume Hood A.* The filter paper should be thrown away in the trash can.

26. Clean and wipe dry your laboratory work area and all apparatus. When you have completed your lab report have the instructor inspect your working area. Your lab report will be stamped if your working area is clean, and your lab report can then be turned in to the instructor.
EXPERIMENT 7B LAB REPORT

Name: ___________________________________________  Student Lab Score: ____________________
Date/Lab Start Time: _______________________________  Lab Station Number: __________________

DATA TABLE

<table>
<thead>
<tr>
<th>Wavelength of Peak Absorbance</th>
<th>.</th>
<th>nm</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>SOLUTIONS</th>
<th>Percent CuSO₄·5H₂O</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solution D</td>
<td>.</td>
<td>%</td>
</tr>
<tr>
<td>Solution C</td>
<td>.</td>
<td>%</td>
</tr>
<tr>
<td>Solution B</td>
<td>.</td>
<td>%</td>
</tr>
<tr>
<td>Solution A</td>
<td>.</td>
<td>%</td>
</tr>
<tr>
<td>Blank</td>
<td>.</td>
<td>%</td>
</tr>
</tbody>
</table>

Slope of Calibration Line | . | %⁻¹ |
Y-Intercept of Calibration Line | . |
Correlation Coefficient | . |

SOLUTIONS

<table>
<thead>
<tr>
<th>SOLUTIONS</th>
<th>Percent CuSO₄·5H₂O</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Crystal Solution</td>
<td>.</td>
<td>%</td>
</tr>
</tbody>
</table>

2 Percentage of KNO₃ in Crystals | . | % |

CALCULATIONS

1. 
2. 

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1. A cuvet becomes dirty with either fingerprints, water spots, or lint. Will this cause the absorbance reading of a Cu(NH\textsubscript{3})\textsubscript{4}^{2+} solution to be greater than or less than it should be? Explain.

2. A student made 5 standard solutions and measured their corresponding absorbance values in a 1.00 cm wide cuvet with a spectrometer set at a wavelength of 525.0 nm to generate the graph below.

(a) The slope of the calibration line is given on the graph. What are the units of the slope?

(b) A sample of solution X was tested, and produced an absorbance reading of 0.274. Calculate the mass percent of X in the sample.

(c) Calculate the extinction coefficient for X at 525 nm.